REMARKS

Claims 43 and 44 have been amended without prejudice or disclaimer and without acquiescing to the rejections. Amended claims 43 and 44 recite that the subject antigen presenting cell is a dendritic cell obtained from a monocyte by plastic-adherence followed by culture with GM-CSF and IL-4. Support can be found in paragraph [0054] of the Applicants' specification.

In light of the amendment of claim 43, dependent claims 45 and 47 have been cancelled. Claim 46 has been amended to recite dependency directly to claim 43.

Claims 43 and 45-47 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Buseyne et al. The Applicants respectfully submit that the rejection is now moot with respect to cancelled Claims 45 and 47.

In light of the amendment of claim 43, the Applicants respectfully assert that Buseyne et al. does not anticipate those rejected claims. Amended claim 43 states that the dendritic cell is obtained from a monocyte by plastic-adherence followed by culture with GM-CSF and IL-4. However, the experimental methods described by Buseyne et al. specify that dendritic cells were prepared by culture of monocytes and lymphocytes in GM-CSF and IL-13 and were isolated by elutriation.

Buseyne et al. teaches cell culture with IL-13 treatment, as opposed to IL-4 as recited in the rejected claims. While IL-4 and IL-13 are related proteins, a post-filing publication by Ahn et al. demonstrates that these proteins do not possess the same functionality. Indeed, the publication states that IL-4 is more effective than IL-13 for differentiation of dendritic cells from PBMCs *in vitro* to the dendritic cell phenotype and functionality (See enclosed Ahn et al. (2005) Int. Immuno. 17(10):1337-1346). Their data further shows that mature dendritic cells treated

with IL-4 possess a slightly higher capacity for stimulating autologous T-cell proliferation than those treated with IL-13, although the difference was not statistically significant. Therefore, as the rejected claims recite a different and functionally distinct cytokine (which also produces unexpected and superior results), the teaching of the use of IL-13 in Buseyne et al. does not anticipate the rejected claims.

Furthermore, Buseyne et al. teaches isolation of dendritic cells by elutriation, rather than plastic adherence as recited in those rejected claims. The Applicants respectfully submit that the isolation of dendritic cells by plastic-adherence results in a product that is distinct from the product obtained in Buseyne et al., thus the claims are distinguished over Buseyne et al.

According to the teachings of Buseyne et al., dendritic cells are isolated from a combined culture of lymphocytes and monocytes. However, cytokine treatment of a culture from an HIV-positive sample would stimulate lymphocytes to produce the virus. The entire culture, including the newly differentiated dendritic cells, would be subsequently infected with the patient's active viral strain.

Contrarily, the isolation of dendritic cells by the process recited in the rejected claims yields cells with little or no infection. The monocyte-derived dendritic cells obtained by plastic adherence are not infected because monocytes are, at best, only weakly infected even in an HIV-positive patient. As there are no lymphocytes in the original culture, the culture is free of cells that are likely to produce the virus upon cytokine treatment. Consequently, the virus-free dendritic cells isolated by plastic adherence are suitable for treatment with the AT-2 inactivated virus and injection into the source organism.

When considering the patentability of a product-by-process claim over prior art, the structure implied by the process steps should be considered, especially where the product can

only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product. Based on the foregoing, the Applicants respectfully submit that the manufacturing process steps impart distinctive structural characteristics to the final product, namely, the degree of viral infection of the dendritic cells isolated by the different processes. The distinctive structural characteristics distinguish a product suitable for administration as a pharmaceutical composition from one comprised of cells infected with the active HIV. Accordingly, Buseyne et al. neither anticipates the product-by-process aspect of the rejected claims nor discloses a product suitable for use in the recited pharmaceutical composition. Accordingly, the Applicants respectfully submit that one skilled in the art would not interpret Buseyne et al. as disclosing the product-by-process recited claim 43.

Additionally, the Applicants maintain that Buseyne et al. does not teach an autologous dendritic cell. An autologous cell is harvested from an organism and later administered to the same source organism. Buseyne et al. does not teach *in vivo* protocol or administration of treated dendritic cells to the source organism. Therefore, the dendritic cells described therein are not considered autologous.

For the reasons stated above, the Applicants respectfully submit that Buseyne et al. does not disclose each and every element recited in claim 43, and thus does not anticipate the rejected claims. Accordingly, the Applicants request removal of the rejections of claims 43 and 46 under 35 U.S.C. §102(b) over Buseyne et al.

Claims 52-56 stand rejected under 35 U.S.C. §103(a) as allegedly obvious over Buseyne et al. in view of Lu et al. Specifically, the rejection states that Buseyne et al. teaches a composition comprising an antigen-presenting cell pulsed with an 2,2'-dithiopyridine (AT-2)

inactivated non-recombinant human immunodeficiency virus (HIV) and a pharmaceutically acceptable carrier. The rejection further relies on Lu as allegedly teaching the use of an adjuvant, indinavir, in a non-antiviral concentration for optimizing the virus-specific CTL response.

For the reasons discussed above, the Applicants respectfully submit that Buseyne et al. neither teaches nor suggests a dendritic cell obtained from a monocyte by plastic-adherence followed by culture with GM-CSF and IL-4, as recited in amended claim 43 and further embodied in the rejected dependent claims 52-56. Lu et al. teaches only the use of indinavir to enhance survival and proliferation of T-cells and fails to cure the deficiency of Buseyne et al. with respect to plastic-adherence and IL-4 treatment. Accordingly, the hypothetical combination of Buseyne et al. and Lu et al. fails to teach or suggest each and every element recited in the rejected claims. Thus, claims 52-56 are non-obvious in view of these documents and the Applicants respectfully request removal of the rejections claims 52-56 under 35 U.S.C. 103(a) over Buseyne et al. in view of Lu et al.

Claim 44 stands rejected under 35 U.S.C. 103(a) as allegedly obvious over Buseyne et al. in view of Lieberman et al. Specifically, the rejection states that Buseyne teaches a composition comprising an antigen-presenting cell pulsed with an 2,2'-dithiopyridine (AT-2) inactivated non-recombinant human immunodeficiency virus (HIV) and a pharmaceutically acceptable carrier. The rejection further relies on Lieberman et al. as teaching that mutation of the epitope recognized by HIV-specific CTL can side-step CTL recognition.

As discussed above, the Applicants assert that Buseyne et al. neither discloses nor suggests a composition comprised of a dendritic cell obtained from a monocyte by plastic-adherence followed by culture with GM-CSF and IL-4, as recited in amended claim 44.

Furthermore, Lieberman et al. merely notes that viral mutation is one mechanism by which the human immunodeficiency virus evades CTL recognition. Lieberman et al. does not suggest a pharmaceutical composition designed to counteract the problem or, further, a pharmaceutical composition comprised of an autologous HIV strain. Lieberman et al. also fails to cure the deficiency of Buseyne et al. with respect to plastic-adherence and IL-4 treatment. Accordingly, the hypothetical combination of Buseyne et al. and Lieberman et al. fails to teach each and every element recited in the rejected claim. The subject matter of rejected claim 44 is non-obvious in view of Lieberman et al., and removal of the rejection of claim 44 under 35 U.S.C. 103(a) over Buseyne et al. in view of Lieberman et al. is respectfully requested.

Claims 43-47 and 52-56 are provisionally rejected on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claim 1 of co-pending Application No. 11/138,171 in view of Buseyne et al., Lu et al., and Lieberman et al.

Claims 43-47 and 52-56 are further provisionally rejected on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 2, 7, and 13 of co-pending Application No. 11/243,094 in view of Buseyne et al., Lu et al., and Lieberman et al.

As the above rejections are provisional, the Applicants will address the rejections upon indication of otherwise allowable claims.

In light of the foregoing, the Applicants respectfully submit that the application is in condition for allowance, which is respectfully requested.

Respectfully submitted,

T. Daniel Christenbury
Registration No. 31,750

TDC/LL/sh
(215) 656-3381

International Immunology, Vol. 17, No. 10, pp. 1337–1346
doi:10.1093/intimm/dxh312

IL-4 is more effective than IL-13 for *in vitro* differentiation of dendritic cells from peripheral blood mononuclear cells

Justin S. Ahn and **Babita Agrawal**

Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2S2, Canada

Keywords: cytokines, dendritic cells, immune response, T lymphocytes

Abstract

Dendritic cells (DCs) are the most potent professional antigen-presenting cells which can activate T cells to induce the primary immune response. For clinical studies, DCs are often differentiated *in vitro* from peripheral blood mononuclear cells (PBMCs) through treatment with granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4. However, IL-13, a cytokine closely related to IL-4, has also been reported to induce differentiation equally or more efficiently when used with GM-CSF. For the present study, we compared the DC characteristics exhibited by iDCs and LPS-matured DCs differentiated from PBMCs using GM-CSF and IL-4 or IL-13. Physical characteristics examined include cellular morphology and surface phenotype. Functional traits investigated include FITC–dextran uptake, IL-10 and IL-12 production, allostimulation and cytokine production by stimulated T cells and antigen-specific T cell stimulation. Compared with IL-13-derived DCs, IL-4 treatment yielded more differentiated DCs, with extensive dendrites and higher expression of DC-SIGN, DEC-205, CD86 and HLA-DR. In addition, IL-4 DCs were more efficient at inducing allogeneic T cell proliferation and immature IL-4 DCs had higher endocytic activity at low FITC–dextran concentrations (1 μg ml^{-1}). Although IL-13 was capable of generating DCs from PBMCs, it was not as effective as IL-4 in generating DC phenotype and functionality. Thus, the use of GM-CSF and IL-4 is the more efficient treatment for inducing DC differentiation from PBMCs.

Introduction

Dendritic cells (DCs) are the most potent professional antigen-presenting cells which play a critical role in activating T cells to initiate the adaptive immune response. Immature dendritic cells (iDCs) efficiently recognize and take up foreign material in the tissues and blood and then process and present antigens on their surface in the context of MHC molecules. With appropriate danger signals, mature dendritic cells (mDCs) will stimulate $CD4^+$ and $CD8^+$ T cells to mount a humoral and/or cell-mediated immune response against the targeted pathogen (1). Due to their fundamental role in initiating hosts' cellular immunity, DCs have been the recent focus of immunotherapeutic research as potential cellular vaccines against malignant tumors and viral infections such as hepatitis C and HIV (2, 3).

Since DCs comprise only ~0.3% of peripheral blood mononuclear cells (PBMCs), it is common to use *in vitro* techniques to generate DC cultures (3, 4). The most common technique differentiates DCs from adherent PBMCs using IL-4 and granulocyte macrophage colony-stimulating factor (GM-CSF). This treatment has been documented to provide good *in vitro* yields of cells bearing phenotypic and functional DC characteristics (5, 6). However, some groups have reported that IL-13 plus GM-CSF can also differentiate PBMCs into DCs equally (7, 8) or more efficiently (4) than IL-4 plus GM-CSF.

IL-13 is a pleiotropic cytokine secreted by activated T_h2 cells (9, 10), activated B cells (11), basophils (12, 13), mast cells (14, 15) and phorbol myristate acetate-ionomycin-stimulated DCs (16). The cytokine shares strong homology with IL-4. Both cytokines have ~30% amino acid homology, similar intron–exon structures and chromosomal locations (10, 17). In addition to structural similarities, they also exhibit functional overlap. Both cytokines induce IgE class-switching and CD23 expression in B cells (11, 18, 19), are involved in antigen-induced airway eosinophilia and airway hyperresponsiveness (20) and inhibit pro-inflammatory cytokine secretion from activated macrophages by regulating secretion of IL-10 (9, 21–23). The most significant difference is that IL-4 is necessary for $CD4^+$ T_h2 cell development, while IL-13 has

Correspondence to: B. Agrawal; E-mail: bagrawal@ualberta.ca

Transmitting editor: A. Falus

Received 18 July 2005, *accepted* 22 July 2005

Advance Access publication 2 September 2005

been reported to have negligible effects on T cells (24, 25). However, McKenzie *et al.* (26) has implicated that IL-13 in mice has a possible immunoregulatory role and may affect T_h2 cell development. Functional differences may exist because while the IL-13R and IL-4R share a common IL-4Rα-signaling chain, they also have distinct receptor subunits as well (24, 25). IL-4Rα can dimerize with the common cytokine γc chain (type I receptor) or with the IL-13Rα chain (type II receptor). IL-4 can bind and function through both receptors but IL-13 can only bind to the type II receptor (27, 28). In addition, IL-4 antagonists have been documented to interfere with IL-13 activities (20, 29) and IL-13 antagonists have been reported to partially inhibit IL-4 activities (30), augmenting the close relationship between these two cytokines. If IL-13 proves to be more effective than IL-4, then it would help researchers to optimize DC cultures from limited quantities of patient's blood.

For our study, we compared the DC traits exhibited by iDCs and LPS-matured DCs differentiated from PBMCs using GM-CSF and IL-4 or IL-13. Our objective was to determine which cytokine was more effective at differentiating DCs from peripheral blood monocytes. We assessed the degree of differentiation through analysis of physical and functional DC characteristics, including cellular morphology, surface phenotype, FITC–dextran uptake, IL-10 and IL-12 production, stimulation of allogeneic T cell proliferation and cytokine secretion and tetanus toxoid (TT) antigen-dependent autologous T cell stimulation. Substituting IL-4 with IL-13 for *in vitro* DC differentiation was particularly interesting because IL-13 has negligible effects on T cells and, therefore, DCs generated in the presence of IL-13 for clinical vaccinations would not have the risk of directly modulating the T cell response in case of carryover of culture media.

Methods

In vitro generation of DCs from human blood

Peripheral blood samples were obtained from donors 30–60 years of age of both sexes after informed consent. Use of human blood samples was approved by the institutional Health Research Ethics Board at the University of Alberta, Canada. Blood obtained from human donors was partitioned overtop of lymphocyte separation medium (Cellgro, Herndon, VA, USA) and centrifuged at 2000 r.p.m. for 30 min. The intermediate buffy layer containing PBMCs was extracted, washed twice in warm PBS and then re-suspended in DC media: RPMI 1640 supplemented with 1% penicillin–streptomycin, 1% sodium pyruvate (Invitrogen, Carlsbad, CA, USA) and 1% human AB serum (Sigma, St Louis, MO, USA). PBMCs were seeded into 6-well plates at 3×10^7 cells per well and then incubated for 2 h in a humidified chamber at 37°C and 5% CO_2 (all incubations were done under these conditions and will hereafter be referred to as 37°C). Non-adherent cells were subsequently removed (typically ~75–80% of PBMCs) and cryopreserved in 90% FBS (Invitrogen) and 10% dimethyl sulfoxide (Sigma) for future assays. Remaining adherent cells were supplied with 5 ml of fresh DC media and the corresponding cytokine treatments: 50 ng ml^{-1} GM-CSF and 10, 20 or 100 ng ml^{-1} of IL-4 or IL-13 (Peprotech, Ottawa, ON, Canada). The optimum concentration determined for both ILs was 10 ng ml^{-1} and was used for subsequent DC differentiation. After 6 days of incubation at 37°C, DC cultures were incubated with 1 μg ml^{-1} LPS (Sigma) for 18 h to induce maturation. Before cells were harvested for experiments, 100 μl of supernatant was collected from each well and frozen for cytokine analysis. Cell viability was assessed using trypan blue staining and hemocytometer counts.

Cellular morphology analysis

The morphology of the cells was examined on day 6, ~5 h after LPS stimulation using a phase contrast microscope and digital photography to record the images.

Surface antigen expression

Cell suspensions containing 3×10^5 to 4×10^5 DCs were stained for 30 min on ice with 5 μl of one of the following mAbs: anti-HLA-DR (QR), MHC class I (FITC) and CD14 (FITC) from Sigma; anti-CD40 (PE), CD80 (PE–Cy5) and DEC-205 (FITC) from eBioscience (San Diego, CA, USA); anti-DC-SIGN (APC) from R&D Systems (Hornby, ON, Canada) and anti-CD11c (PE), CD80 (PE), CD86 (PE), CCR5 (PE–Cy7), CCR (PE–Cy7) and isotype control mouse IgG (FITC, QR, PE, APC, PE–Cy5 and PE–Cy7) from Becton Dickinson (Mississauga, ON, Canada). Cells were washed twice with cold PBS + 1% sodium azide + 2% FBS (FACS wash) and then fixed with PBS + 1% sodium azide + 1% PFA (FACS fix). Samples were analyzed using FACSScan or FACSCalibur and Cell Quest software (Becton Dickinson).

FITC–dextran uptake assay

Cell suspensions containing 4×10^5 DCs were incubated on ice (4°C) for 30 min prior to the assay. Cells were centrifuged, re-suspended in a small volume of DC media and treated with FITC–dextran (Sigma) at final concentrations ranging from 1 μg to 1 mg ml^{-1}. Cells were incubated at 37 or 4°C for 1 h and then both groups were incubated at 4°C for an additional 30 min. Cells were washed four times with FACS wash and then fixed with FACS fix. Samples were analyzed using FACSScan and Cell Quest software.

IL-10, IL-12, tumor necrosis factor-α and IFN-γ secretion

Detection of secreted cytokines was carried out using IL-10, IL-12, tumor necrosis factor (TNF)-α and IFN-γ sandwich ELISA kits purchased from Biosource International (Camarillo, CA, USA). The assay was performed as outlined in the instructions. Sample dilutions were assessed after varying trials and ranged from 1:20 to 1:4. The standards ranged from 15.125 to 2000 pg ml^{-1}.

Allogeneic T cell proliferation

DCs were washed to minimize carryover of IL-4 or IL-13 in the media, re-suspended in AIM-V medium (Invitrogen) and then added to allogeneic T cells in triplicates at ratios of 1:4, 1:10, 1:20, 1:40, 1:100 and 1:200 (DCs to T cells). Controls included wells with only media, only DCs, only T cells and T cells plus 1 μg ml^{-1} PHA (Sigma). After 4 days of incubation at 37°C, 60 μl of media was carefully collected for cytokine analysis and then the wells were incubated with 0.5 μCi ml^{-1} [^{3}H]thymidine

([^{3}H]TdR) (Amersham, Piscataway, NJ, USA) for 18 h, harvested and analyzed using a microbeta liquid scintillation counter (Wallac).

TT-specific autologous T cell proliferation

iDCs were harvested on day 6, re-suspended in AIM-V and then cultured in triplicates into 96-well plates at 1×10^3, 2×10^3, 5×10^3, 1×10^4 and 2×10^4 cells per well. A total of 0.5 $\mu g\ ml^{-1}$ of TT (Sigma) was added to the experimental wells only. DCs were incubated with TT for 4 h at 37°C. DCs were treated with 1 $\mu g\ ml^{-1}$ LPS and then incubated for an additional 18 h at 37°C. The next day, 2×10^5 autologous T cells were added to all wells except for the media and DC-only controls. Other controls included T cells only, T cells plus 1 $\mu g\ ml^{-1}$ PHA and T cells plus 0.5 $\mu g\ ml^{-1}$ TT. After 5 days of incubation at 37°C, wells were incubated with 0.5 $\mu Ci\ ml^{-1}$ [^{3}H]TdR for 18 h, harvested and analyzed with a microbeta liquid scintillation counter.

Statistical analysis

Student's *t*-test was performed for cytokine and proliferation assays using SPSS v.13 software.

Results

IL-4 treatment generates more pronounced DC morphology

The combination of GM-CSF and IL-4 or IL-13, after 6–7 days of culture yielded between 2.25×10^6–4.45×10^6 cells per well in various experiments representing between 38–75% yields (data not shown). There was no significant difference between the two treatment groups with respect to cell yield. The viability for all of the experimental groups was similar at >80% (data not shown). Under the phase contrast microscope, we found that the majority of IL-4 DCs showed characteristic DC morphology: large semi-adherent cells growing in clumps and exhibiting long cytoplasmic protrusions (Fig. 1A). Conversely, only a minority of IL-13 iDCs exhibited dendrites which were also sparse and poorly developed (Fig. 1B). DCs matured with LPS showed extensive dendrite development and formed fibrous, adherent clumps. Morphology was still more pronounced in the IL-4 mDCs than in the IL-13 mDCs (Fig. 1C and D, respectively).

IL-4-derived DCs have higher expression of HLA-DR and co-stimulatory molecules

For flow cytometry analysis, DCs were initially gated according to their forward- and side-scatter properties. Although the expression of the myeloid DC marker, CD11c, was slightly higher on IL-4 DCs, the percentage of positive cells were similar between the two groups and was usually >90%, thus indicating that the majority of gated cells was indeed DCs (Fig. 2A). Compared with the IL-13-treated DCs, a greater proportion of IL-4-treated iDCs and mDCs expressed HLA-DR and CD86 (Fig. 2A and B). In addition, IL-4 DCs expressed higher levels of the DC-specific C-type lectin, DC-SIGN (31) and the macrophage mannose receptor homolog, DEC-205 (32), which was nearly absent on IL-13 DCs (Fig. 2C). The percentage of positive cells and the mean fluorescence

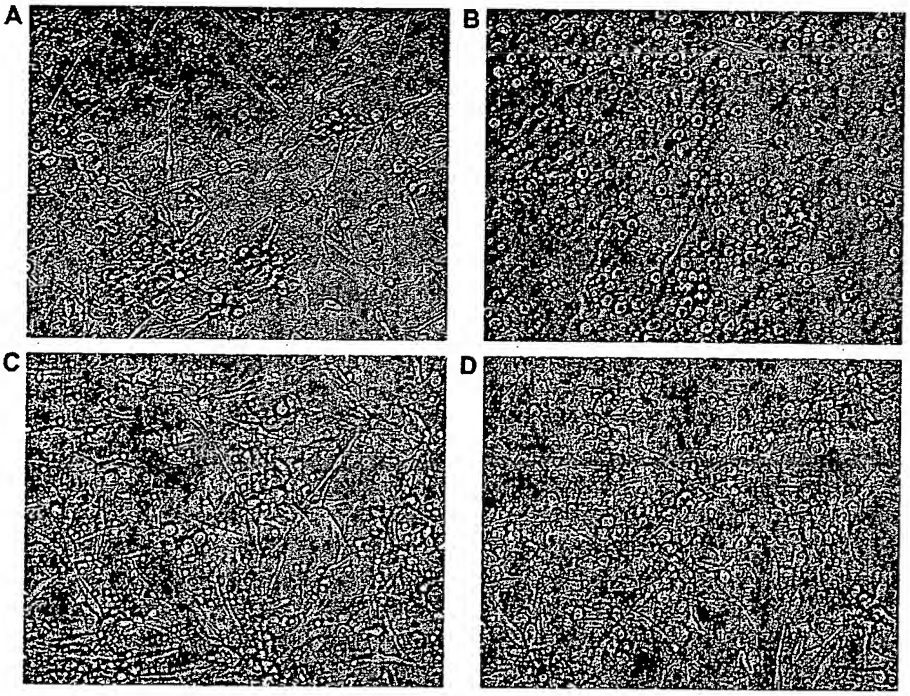


Fig. 1. Morphology of DCs under phase contrast microscopy at ×100. (A) IL-4-derived iDCs (B) IL-13-derived iDCs (C) IL-4-derived and LPS-matured DCs and (D) IL-13-derived and LPS-matured DCs.

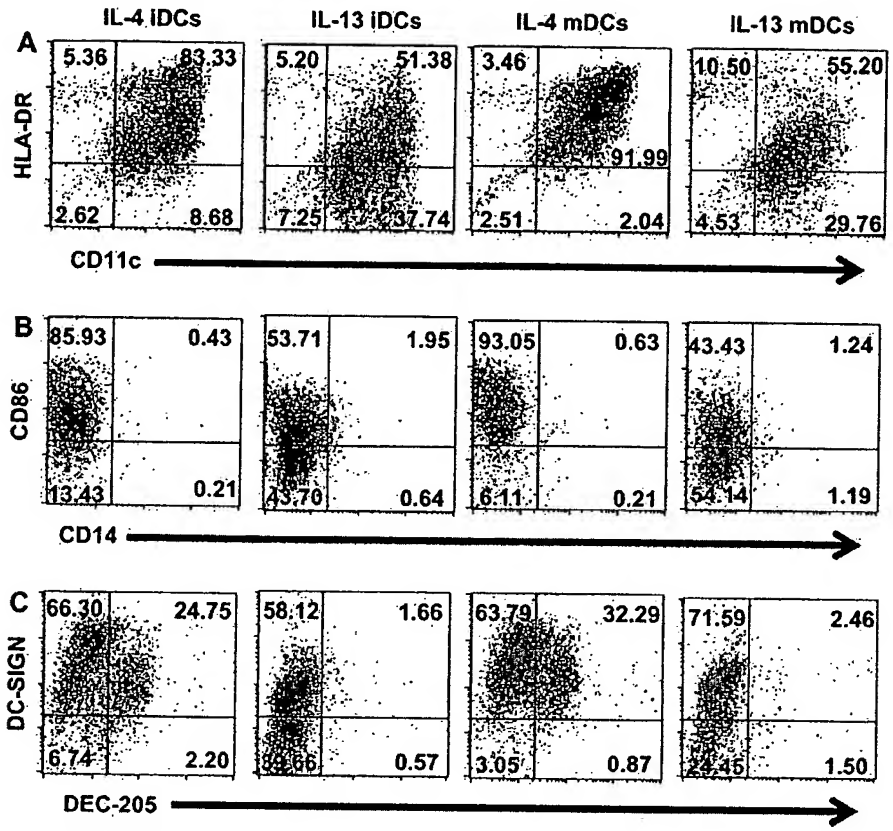


Fig. 2. Surface phenotype of iDCs and LPS-matured DCs differentiated with GM-CSF and IL-4 or IL-13. Markers analyzed include (A) HLA-DR and CD11c, (B) CD86 and CD14 and (C) DC-SIGN and DEC-205. DCs in all groups were gated similarly on the basis of forward and side scatter. Digit in each corner of the dot plots represents the percentage of gated DCs in each quadrant. These data are representative of three independent experiments.

intensity (MFI) of DC markers such as HLA-DR, CD11c, CD86 and CD40 were considerably higher on IL-4-derived DCs than on IL-13-derived DCs (Fig. 3). The chemokine receptors, CCR5 and CCR7, were expressed slightly higher on the IL-13 DCs but the difference was not significant (data not shown). The expression of MHC class I was much more prevalent on the IL-4 DCs than on the IL-13 DCs (Fig. 3). However, there was no notable difference in CD80 and CD83 expression between the two groups (data not shown).

IL-4-derived DCs exhibit greater endocytic capacity at low antigen concentrations

At high FITC–dextran concentrations ($\geq$100 μg ml^{-1}), the two iDC cultures showed similar uptake capacity (Fig. 4A and B). However, when the FITC–dextran concentration was <5 μg ml^{-1}, only IL-4 iDCs were capable of significant uptake (Fig. 4C). At 1 μg ml^{-1} FITC–dextran, only 2.29% of IL-13-derived iDCs were capable of endocytosis compared with 47.53% of IL-4 iDCs (Fig. 4D and E). Thus, IL-13 iDCs do not appear to be efficient at endocytosis at low antigen concentrations. Overall, uptake and, thus, MFI increases as the concentration of FITC–dextran increases. For both DC samples, uptake of FITC–dextran was significantly down-regulated after LPS maturation.

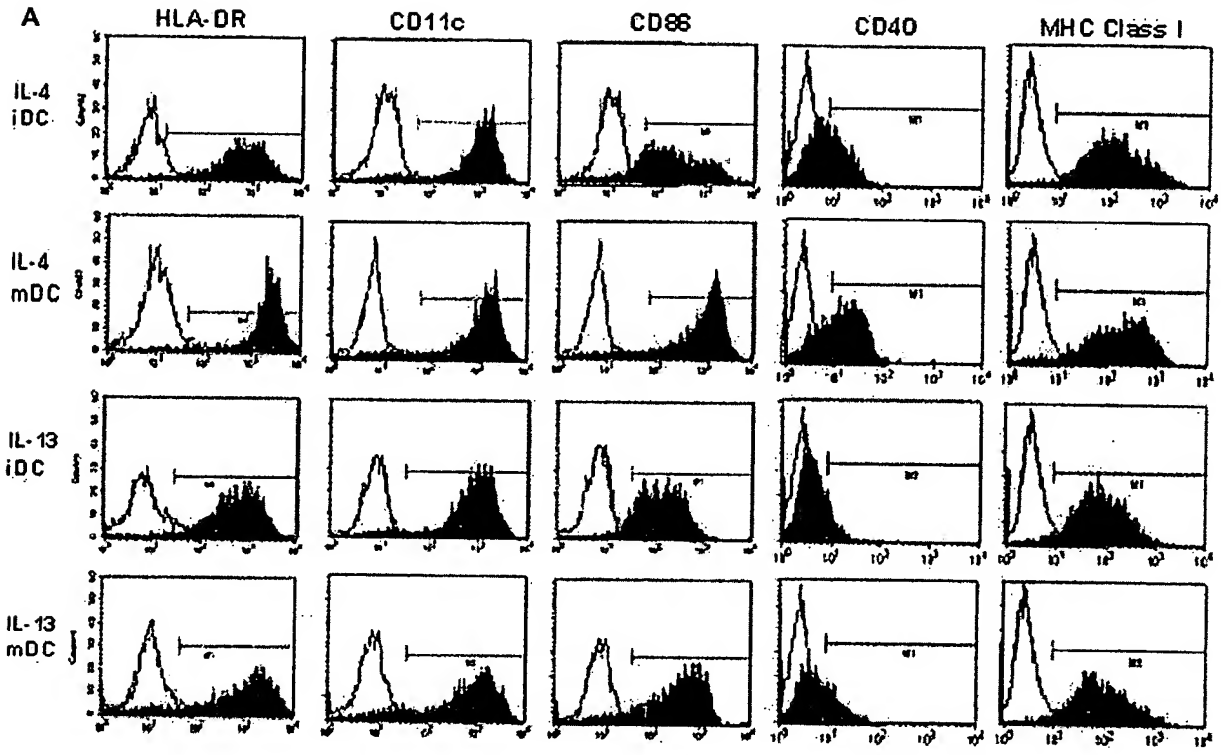
Both IL-4- and IL-13-derived mDCs secrete IL-10 and IL-12

iDCs from both GM-CSF plus IL-4 or GM-CSF plus IL-13-treated cultures did not produce detectable levels of IL-10 (<15 pg ml^{-1}). On the other hand, IL-4 and IL-13 mDCs secreted 4.4 ± 0.3 × 10^3 and 2.9 ± 0.3 × 10^3 pg ml^{-1} of IL-10, respectively (Fig. 5A). However, the difference between the two levels was not significant ($P > 0.05$). In contrast, iDCs differentiated with GM-CSF plus IL-4 or IL-13; both secreted low levels of IL-12. When stimulated with LPS, the IL-13-derived mDCs secreted significantly more IL-12 than the IL-4-derived DCs ($P < 0.01$): 8.4 + 1.1 × 10^4 versus 2.2 + 0.6 × 10^4 pg ml^{-1}, respectively (Fig. 5B).

IL-4-derived DCs are better stimulators of allogeneic T cell proliferation

IL-4-derived mDCs, showed significantly stronger allostimulatory ability than IL-13 DCs ($P < 0.05$). Stimulatory activity was up-regulated upon maturation of the DCs (data not shown). In particular, the T cell proliferation stimulated by IL-4 mDCs reached a peak of 104 ± 4 × 10^3 counts per minute (c.p.m.) (at 1 × 10^4 DCs) compared with only 50 ± 4 × 10^3 c.p.m. (at 1 × 10^4 DCs) with IL-13 DCs (Fig. 6). Controls showed that

A



B

	HLA-DR	CD11c	CD86	CD40	MHC class I
IL-4 iDC	915.24	1310.53	496.80	17.17	232.04
	98.38%	94.46%	81.07%	41.29%	98.74%
IL-4 mDC	2489.21	1590.86	1320.93	24.82	299.01
	98.68%	93.68%	94.04%	69.66%	97.81%
IL-13 iDC	855.53	1106.05	199.43	12.70	123.73
	98.8%	96.29%	90.69%	11.84%	97.05%
IL-13 mDC	1604.33	1336.97	563.45	15.34	148.37
	98.8%	89.78%	91.0%	28.00%	98.21%

Fig. 3. Surface phenotype of iDCs and LPS-matured DCs differentiated with GM-CSF and IL-4 or IL-13. (A) Surface antigen expression by FACS analysis: HLA-DR, CD11c, CD86, CD40 and MHC class I. The shaded histograms represent the stained samples, while the unshaded histograms represent the unstained controls. (B) MFI of the peaks in the histograms in (A) and the proportion of cells expressing fluorescence. DCs in all groups were gated similarly on the basis of forward and side scatter. These data are representative of three independent experiments.

T cells (<6500 c.p.m.) and DCs (<1200 c.p.m.) alone did not proliferate, while T cells stimulated with 1 μg ml^{-1} PHA were capable of proliferation (3 × 10^4 c.p.m.) (data not shown). Proliferation response was dose dependent until 1 × 10^4 DCs, where activity began dropping off in both samples.

Allogeneic T cells stimulated with IL-4-derived DCs secrete greater IFN-γ and less TNF-α than IL-13-derived DCs

To assess polarization of T cell response during allogeneic T cell stiimulation, we collected the supernatant from these assays and tested them for the T_h1 cytokines TNF-α and IFN-γ and the T_h2 cytokine IL-10. DCs alone produced almost no IFN-γ (<8 pg ml^{-1}) and minimal levels of TNF-α (<700 pg ml^{-1}) (data not shown). Overall, TNF-α was the predominant cytokine secreted by T cells in the allostimulation assays, followed by IFN-γ and no detectable levels of IL-10. Incubation with IL-4 or IL-13 iDCs did not enhance TNF-α and IFN-γ secretion, as secretion levels were similar in T cell-only controls. However, T cells stimulated with mDCs greatly up-regulated the cytokine secretion. T cells stimulated with IL-13 mDCs secreted twice as much TNF-α than T cells stimulated with IL-4 mDCs (Fig. 7A). In contrast, T cells stimulated by IL-4 mDCs secreted three times more IFN-γ than T cells stimulated with IL-13 mDCs (Fig. 7B). Although TNF-α appears to be a major cytokine secreted in allostimulation, an elevated level secreted by IL-13 mDC-stimulated

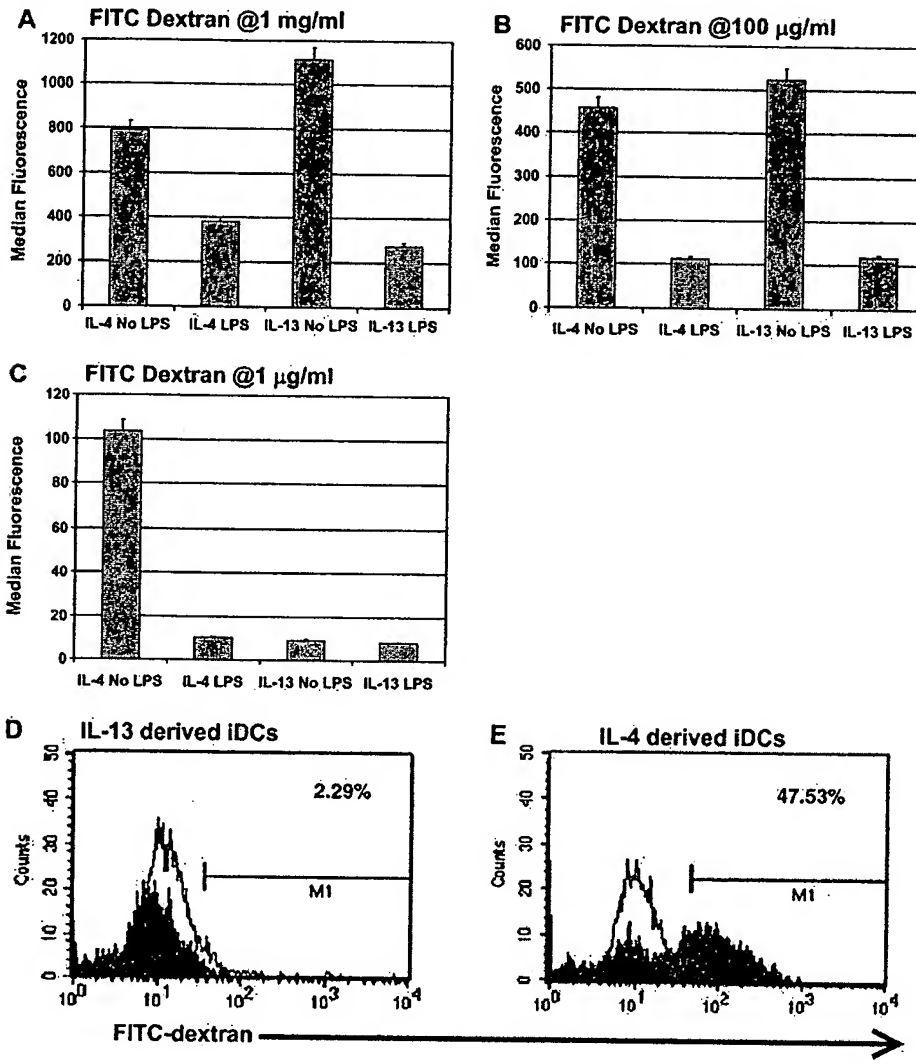


Fig. 4. Endocytosis of FITC–dextran by iDCs and mDCs at 37°C. DCs were treated with GM-CSF and IL-4 or IL-13 LPS was added to induce maturation. FITC–dextran was added at concentrations of (A) 1 mg ml^{-1}, (B) 100 µg ml^{-1} and (C) 1 µg ml^{-1}. FITC–dextran uptake is represented by median fluorescence. (D) Endocytosis of 1 µg ml^{-1} FITC–dextran by iDCs. The fluorescence of FITC–dextran-treated DCs are represented by the shaded histograms. The 4°C control was used to set the background fluorescence and is represented by the unshaded histograms. 2.29% of IL-13. DCs were capable of FITC–dextran uptake. (E) 47.53% of IL-4 DCs were capable of FITC–dextran uptake with a MFI = 139.69.

T cells does not correspond to higher proliferation. In contrast, the IFN-γ levels correlated with increased proliferation of T cells upon stimulation with IL-4 DCs, as compared with IL-13 DCs.

IL-4 and IL-13 DCs induce antigen-dependent autologous T cell proliferation equally

In both groups, mDCs loaded with TT showed stronger autologous T cell proliferation capability than iDCs. Cultures without TT addition did not exhibit strong T cell proliferation, so the T cell proliferation we observed is due to processing and presentation of TT by DCs (data not shown). Although IL-4 mDCs showed slightly higher stimulatory capacity than IL-13 mDCs, this difference was not significant ($P > 0.05$) (Fig. 8). Similar to the allostimulation results, antigen-dependent autologous T cell stimulation also appears to be dose dependent but begins to taper off ~1 × 10^4 DCs. However, the degree of [^{3}H]TdR incorporation was significantly lower compared with the allostimulation. In the controls, TT alone had minimal proliferative effect on T cells (6603 c.p.m.), T cell and DC-only controls showed minimal proliferation (<2000 c.p.m.).

Discussion

The DCs differentiated from adherent monocytes with GM-CSF and IL-4 or IL-13 exhibited characteristic DC morphology, surface phenotype and function. Stimulation with LPS yielded mDC phenotype including significant up-regulation of HLA-DR, CD11c and CD86, reduced antigen uptake capacity, amplified IL-10 and IL-12 secretion and stronger stimulation of allogeneic and antigen-dependent autologous T cells. However, except IL-12 production, IL-4-derived DCs exhibited

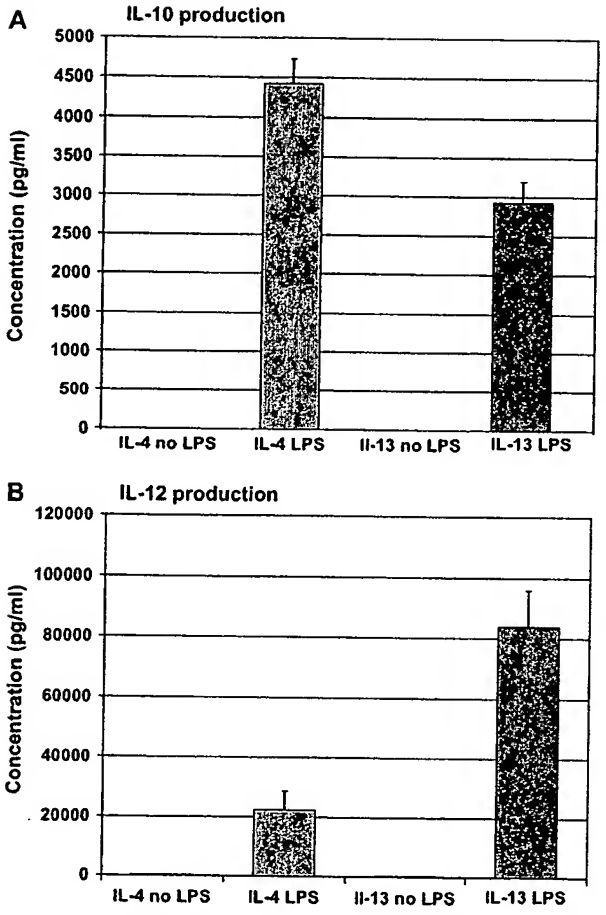


Fig. 5. Cytokine secretion by DCs differentiated with GM-CSF and IL-4 or IL-13 and treated with or without LPS. (A) iDCs did not secrete any detectable levels of IL-10. IL-4-derived mDCs secreted slightly more IL-10 than IL-13-derived mDCs. Graphs were compiled from the averages of two independent samples. (B) iDCs secreted low levels of IL-12. IL-13-derived mDCs secreted significantly more IL-12 than IL-4-derived mDCs. Graphs were compiled from the averages of four independent samples.

more pronounced DC traits than IL-13-derived DCs, leading to the conclusion that IL-4 is more effective than IL-13 at differentiating DCs *in vitro*.

Microscopic analysis revealed that DCs differentiated with IL-4 and GM-CSF exhibited prominent dendrite development in both iDC and mDC cultures. In contrast, the IL-13-derived DCs were poorly differentiated, even upon LPS stimulation. The morphological differences were accompanied by stronger expression of HLA-DR, CD86, DC-SIGN, DEC-205, and MHC class I CD40 on the IL-4 DCs. Despite this, strong CD11c and weak CD14 expression in both subgroups, in conjunction with appropriate side- and forward-scatter properties, confirmed that both subsets represented differentiated DCs. However, reduced dendrite development and lower surface expression of key DC markers on IL-13-derived DCs may compromise some of their functional characteristics. In contrast, the slightly higher CCR5 and CCR7 expression on IL-13 DCs suggests that they may possess greater migratory activity than IL-4 DCs (33).

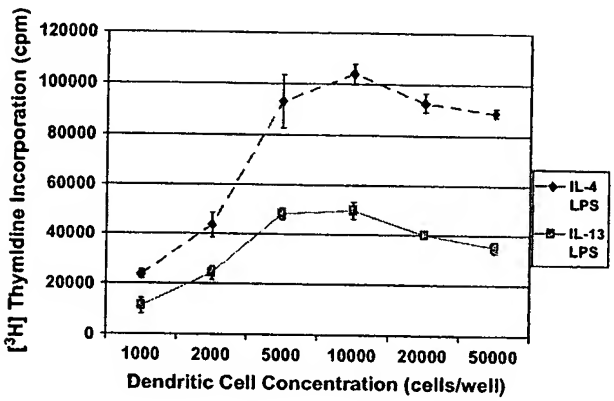


Fig. 6. Allogeneic T cell proliferation by mDCs differentiated with GM-CSF and IL-4 or IL-13 and matured with LPS. Proliferation was represented by [^{3}H]TdR incorporation. T cells were cultured with allogeneic DCs for 4 days and then pulsed with [^{3}H]TdR for 18 h before harvesting. Proliferation is represented as the mean counts per minute ± SD for triplicate cultures.

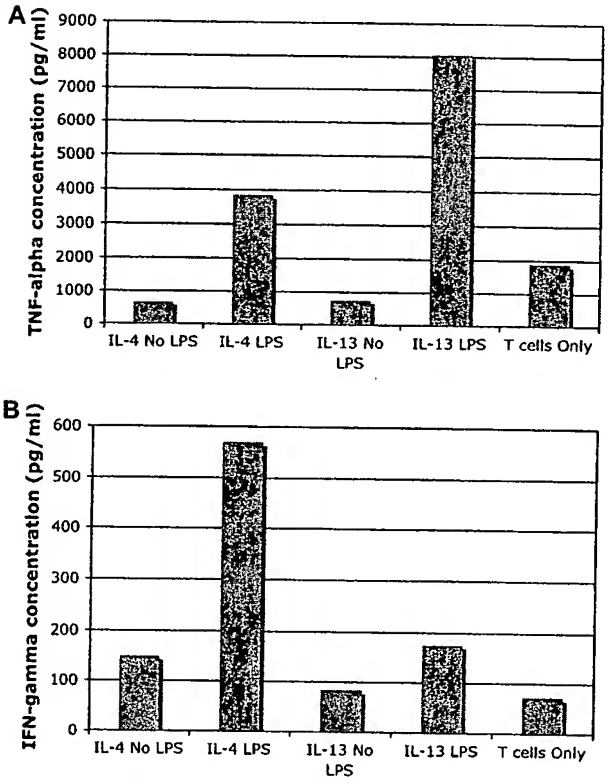


Fig. 7. Cytokine secretion by T cells stimulated with allogeneic DCs differentiated with GM-CSF and IL-4 or IL-13 and matured with LPS. (A) T cells stimulated with IL-13 mDCs secreted greater TNF-α than T cells stimulated with IL-4 mDCs. (B) T cells stimulated with IL-4 mDCs secreted greater IFN-γ than T cells stimulated with IL-13 mDCs. For both graphs the data are representative of two individual experiments where a ratio of 1:40 (DCs:T cells) was used during the stimulation.

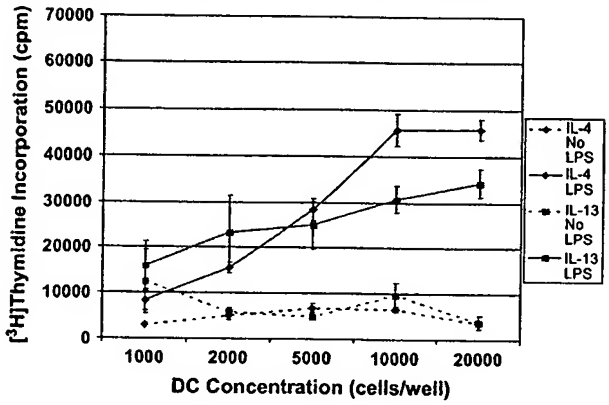


Fig. 8. TT-dependent autologous T cell proliferation induced by iDCs or mDCs. DCs were differentiated with GM-CSF and IL-4 or IL-13 and matured with LPS. T cells were cultured with autologous DCs for 5 days with 0.5 μg ml^{-1} TT and then pulsed with [^{3}H]TdR for 18 h before harvesting. Proliferation is represented as mean counts per minute ± SE for triplicate cultures.

However, it is difficult to assess the migratory function of DCs *in vitro* due to the number of variables involved, such as IL-10 which has been proposed to generate functional decoys that prevent mature chemokine receptor expression on DCs (34).

The responsibilities of DCs are to take up and process exogenous antigens and then present them to T cells on MHC molecules to initiate the immune response (1). In our study we compared the functional capabilities of both iDCs and mDCs from two sub-populations at various steps, starting with endocytosis. FITC–dextran uptake occurs via mannose receptor-mediated endocytosis, a mechanism that is similar to exogenous antigen uptake (4, 5). In both DC subsets, iDCs showed strong endocytic activity which was significantly down-regulated upon LPS stimulation. However, the immature IL-4 DCs demonstrated greater uptake at low FITC–dextran concentrations (1 μg ml^{-1}), suggesting that they are more proficient at recognizing and endocytosing smaller quantities of antigens than IL-13-derived DCs. This is a relevant discovery because low antigen levels may be more reflective of *in vivo* infections and the ability to efficiently detect and present minute amounts of foreign antigen would likely enhance the kinetics of the immune response. Langenkamp *et al.* (35) has shown that the kinetics of DC priming is important in determining the T_h1 or T_h2 T cell response. Thus, efficient uptake and presentation may help the immune system clear pathogens faster by initiating the appropriate immune response and preventing them from establishing and sustaining sufficient populations. However, future studies will need to examine the kinetics of immune responses corresponding to the antigen concentration and DC differentiation technique. Interestingly, the higher sensitivity to low antigen concentration correlates to stronger DEC-205 expression and longer and more developed dendrites present on IL-4 iDCs, compared with IL-13 iDCs. A possible reason why uptake between the two sub-populations is similar at higher FITC–dextran concentrations is because the receptors may become oversaturated at high concentrations.

DCs secrete a wide variety of cytokines, both constitutively and upon stimulation (16). IL-10 is secreted primarily by PBMC-derived-mDCs and germinal center DCs (16, 23) and is a key immunoregulator with proposed functions in controlling T cell activation (23) and inducing T cell anergy (36). In addition, Kambayashi *et al.* (21) demonstrated that IL-4 and IL-13 stimulated IL-10 production in DCs which inhibited secretion of TNF-α and IL-6. However the suppressive effects of IL-10 have been shown to be similar but independent to the cytokine-suppressive effects of IL-13 and IL-4 in activated monocytes (25, 37). Our assay showed that IL-4 and IL-13-derived DCs secreted similar levels of IL-10, suggesting that IL-10 does not account for the differences in T cell stimulation observed between the experimental groups. On the other hand, IL-12 secretion, a hallmark of DC maturation, was significantly greater with the IL-13 DCs than the IL-4 DCs. These data are contrary to those of Lutz *et al.* (27) who found that in murine bone marrow-derived DCs, IL-4 nearly doubled the secretion of IL-12, whereas IL-13 had no effect. However, the effects of IL-4 and IL-13 on murine DCs are not clear and differ from human DCs (28). Ria *et al.* (38) has shown that exogenous IL-10 is capable of inhibiting T_h1 cell-mediated IL-12 production by DCs. Thus, it may be possible that IL-4 DCs are more sensitive to the regulatory effects of IL-10, but this is yet to be determined. It is also possible that other exogenous cytokines may have stimulating or inhibitory effects that could account for the differences observed between the treatments.

The ability of DCs to induce T cell proliferation was tested using both allogeneic and antigen-dependent autologous T cell-stimulation assays. Stimulation of allogeneic T cells by immature and mature IL-4 DCs were significantly greater than their respective IL-13 DCs. This suggests that the IL-4 differentiates DCs with stronger stimulatory capacity and would thus be more valuable for *in vitro* studies of T cell activation and for potential use in cellular vaccines. The strong allostimulatory ability directly correlates to the enhanced physical characteristics of IL-4 DCs such as profound dendrite development and HLA-DR and co-stimulatory molecule expression, which has been shown to be important for T cell activation *in vivo* (39, 40). Using a Rho inhibitor, Kobayashi *et al.* (41) correlated the loss of dendrites with lower alloantigen-presenting capacity. Similarly, down-regulating DC differentiation with gangliosides has been shown to reduce the expression of MHC class II and co-stimulatory molecules, which consequently impair endocytosis and T cell proliferation (42). However, in contrast to T cell proliferation, the cytokine profile of the T cells in the assay was less distinct. T cells stimulated by IL-4 mDCs secreted more IFN-γ but less TNF-α than T cells stimulated by IL-13 mDCs. While TNF-α was the dominant cytokine produced by proliferating allo-T cells, the magnitude of proliferation paralleled IFN-γ secretion patterns. Secretion of TNF-α, a well-known pro-inflammatory cytokine and endogenous activator of DCs, may further enhance the degree of maturation, particularly of IL-13 mDCs and thus increase their stimulatory capacity. However, even with possible enhanced maturation, the IL-13 mDCs still do not possess the same potency as IL-4 mDCs, evident by their superior ability to induce T cell proliferation and skewed production of the key T_h1 cytokine, IFN-γ.

With the antigen-dependent autologous T cell stimulation, mDCs and iDCs were loaded with TT prior to exposure to T cells. The mDCs showed significantly more stimulatory

capacity than the iDCs and the TT-loaded mDCs showed significantly more stimulatory capacity than the unloaded mDCs. Although T cell proliferation induced by the IL-4 DCs was higher than the IL-13 DCs, there was no significant difference between the two subsets. These results may contrast the allostimulation results due to the lower frequency of TT-reactive T cells than alloreactive T cells used in the stimulation assays.

Sato *et al.* (4) found that IL-13-differentiated DCs were more mature than IL-4-derived DCs and stated that IL-13 can even be used as a maturing agent for IL-4-derived DCs. Conversely, we found that IL-13 DCs were less differentiated than IL-4-derived DCs and exhibited reduced DC characteristics such as endocytosis, surface antigen expression and T cell stimulatory capacity (5, 6). However, differences also exist between the methods of maturation. Goxe *et al.* (43) described variations in DC surface expression of MHC class I, CD40, CD80 and CD86 depending on the type of maturation stimuli used. Thus, future studies should assess whether other maturation agents such as Poly I:C and TNF-α can induce DC characteristics similar to LPS-matured DCs differentiated with GM-CSF and IL-4 or IL-13. For other groups (7, 8), IL-13 was found to be as effective as IL-4 at differentiating DCs but both groups did not assess the matured forms of DCs in all their assays. While we found some important differences between iDCs, we also found significant differences between mDCs in the allostimulation assay. Thus, direct comparison between iDCs and mDCs allowed us to examine all aspects of the DC characteristics, from naive iDCs to mature fully functional DCs.

Although we did not find significant differences in IL-10 secretion and the TT antigen-dependent autologous T cell stimulation, the IL-13 DCs never surpassed the IL-4 DCs in any assay except IL-12 production. In all other assays, the IL-4 DCs demonstrated significantly enhanced morphology, surface antigen expression, antigen uptake capabilities and allo-T cell stimulation than the IL-13 DCs. Thus, IL-4 plus GM-CSF is more efficient than IL-13 plus GM-CSF at differentiating DCs from monocytes *in vitro*. Therefore, IL-13 is not a viable substitute for IL-4 and would not yield optimal DCs for clinical studies. Despite these differences, we cannot ignore the possible role of either cytokine in *in vivo* differentiation of DCs. There are numerous pathways leading to the differentiation of various DC subsets (16). Recently, Roberts *et al.* (44) have demonstrated that DCs from IL-4$^{-/-}$ and wild-type mice bear similar phenotype and ability to stimulate T cells, thus voiding the possible *in vivo* role of IL-4 in DC differentiation. Due to the close relatedness of IL-13 and IL-4 in terms of gene proximity, overlapping functions and homologous receptors, it may be possible that IL-13 is capable of fulfilling some of IL-4's roles but not as effectively. Since IL-4 is not produced by DCs but IL-13 is produced by mDCs differentiated from CD14$^+$ cells, it may be possible that IL-13 is involved in regulating differentiation of DCs in an autocrine manner, while IL-4 is involved in exogenous differentiation of DCs (16). Menetrier-Caux *et al.* (45) has shown that both IL-4 and IL-13 can prevent tumor cells from inhibiting DC differentiation. While we cannot rule out any possible roles of IL-4 and IL-13 in *in vivo* DC differentiation, IL-4 is the more potent cytokine for *in vitro* DC differentiation from monocytes.

Acknowledgements

We are grateful to the Alberta Heritage Foundation for Medical Research (AHFMR, Canada) for a medical scholar award and an establishment grant (B.A.), and to the Canadian Institutes for Health Research (Canada) for an operating grant (EOP 58194) to B.A., for financial support of this research. We thank the Northern Alberta Clinical Trials and Research Centre for providing a summer studentship to J.S.A. We sincerely thank Wen Li, Jie Li and Deepa Kolaseri Krishnadas for their much appreciated assistance in the laboratory. B.A. is a recipient of an AHFMR Medical Scholar Award.

Abbreviations

APC	allophycoerythrin
c.p.m.	counts per minute
DC	dendritic cell
GM-CSF	granulocyte macrophage colony-stimulating factor
[^{3}H]TdR	[^{3}H]thymidine
iDC	immature dendritic cell
mDC	mature dendritic cell
MFI	mean fluorescence intensity
PBMCs	peripheral blood mononuclear cells
Poly I:C	polyriboinosinic polyribocytidylic acid
TT	tetanus toxoid
TNF	tumor necrosis factor
QR	quantum red

References

1 Mellman, I. and Steinman, R. M. 2001. Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106:255.
2 Mohamadzadeh, M. and Luftig, R. 2004. Dendritic cells: in the forefront of immunopathogenesis and vaccine development—a review. *J. Immune. Based Ther. Vaccines* 2:1.
3 Banchereau, J., Schuler-Thurner, B., Paluka, A. K. and Schuler, G. 2001. Dendritic cells as vectors for therapy. *Cell* 106:271.
4 Sato, K., Nagayama, H., Tadokoro, K., Juji, T. and Takahashi, T. A. 1999. Interleukin-13 is involved in functional maturation of human peripheral blood monocyte-derived dendritic cells. *Exp. Hematol.* 27:326.
5 Sallusto, F. and Lanzavecchia, A. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin-4 and downregulated by tumor necrosis factor-alpha. *J. Exp. Med.* 179:1109.
6 Sallusto, F., Cella, M., Danieli, C. and Lanzavecchia, A. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med.* 182:389.
7 Morse, M. A., Lyerly, H. K. and Li, Y. 1999. The role of IL-13 in the generation of dendritic cells *in vitro*. *J. Immunother.* 22:506.
8 Piemonti, L., Bernasconi, S., Walter, L. *et al.* 1995. IL-13 supports differentiation of dendritic cells from circulating precursors in concert with GM-CSF. *Eur. Cytokine Netw.* 6:245.
9 Mckenzie, A. N. J., Culpepper, J., de Waal Malefyt, R. *et al.* 1993. Interleukin-13, a novel T cell-derived cytokine that regulates human monocyte and B cell function. *Proc. Natl Acad. Sci. USA* 90:3735.
10 Minty, A., Chalon, P., Derooq, J. M. *et al.* 1993. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* 362:248.
11 Hajoui, O., Janani, R., Tulic, M. *et al.* 2004. Synthesis of IL-13 by human B lymphocytes: regulation and role in IgE production. *J. Allergy Clin. Immunol.* 114:657.
12 Brunner, T., Heusser, C. H. and Dahinden, C. A. 1993. Human peripheral blood basophils primed by interleukin-3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J. Exp. Med.* 177:605.

13 Li, H., Sim, T. C. and Alam, R. 1996. IL-13 released by and localized in human basophils. *J. Immunol.* 156:4833.
14 Burd, P. R., Thompson, W. C., Max, E. E. and Mills, F. C. 1995. Activated mast cells produce interleukin 13. *J. Exp. Med.* 181:1373.
15 Gordon, J. R., Burd, P. R. and Galli, S. J. 1990. Mast cells as a source of multifunctional cytokines. *Immunol. Today* 11:458.
16 De Saint-Vis, B., Fugier-Vivier, I., Massacrier, C. *et al.* 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. *J. Immunol.* 160:1666.
17 Mckenzie, A. N. J., Li, X., Largaespada, D. A. *et al.* 1993. Structural comparison and chromosomal localization of the human and mouse IL-13 genes. *J. Immunol.* 150:5436.
18 Punnonen, J., Aversa, G., Cocks, B. G. *et al.* 1993. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc. Natl Acad. Sci. USA* 90:3730.
19 Cocks, B. G., de Waal, M. R., Calizzi, J. P., de Vries, J. E. and Aversa, G. 1993. IL-13 induces proliferation and differentiation of human B cells activated by the CD40 ligand. *Int. Immunol.* 5:657.
20 Tomkinson, A., Duez, C., Ciesiewicz, G. *et al.* 2001. A murine IL-4 receptor antagonist that inhibits IL-4- and IL-13-induced responses prevents antigen-induced airway eosinophilia and airway hyperresponsiveness. *J. Immunol.* 166:5792.
21 Kambayashi, T., Jacob, C. O. and Strassmann, G. 1996. IL-4 and IL-13 modulate IL-10 release in endotoxin-stimulated murine peritoneal mononuclear phagocytes. *Cell. Immunol.* 1712:153.
22 Chang, W. L., Baumgarth, N., Yu, D. and Barry, P. A. 2004. Human cytomegalovirus interleukin-10 homolog inhibits maturation of dendritic cells and alters their functionality. *J. Virol.* 78:8720.
23 Caux, C., Massacrier, C., Vanbervliet, B., Barthélémy, C., Liu, Y. J. and Banchereau, J. 1994. Interleukin-10 inhibits T cell alloreaction induced by human dendritic cells. *Int. Immunol.* 6:1177.
24 Zurawski, G. and de Vries, J. E. 1994. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol. Today* 15:19.
25 Chomarat, P. and Banchereau, J. 1998. Interleukin-4 and interleukin-13: their similarities and discrepancies. *Int. Rev. Immunol.* 17:1.
26 McKenzie, G. J., Emson, C. L., Bell, S. E. *et al.* 1998. Impaired development of Th2 cells in IL-13 deficient mice. *Immunity* 9:423.
27 Lutz, M. B., Schnare, M., Menges, M. *et al.* 2002. Differential functions of IL-4 receptor types I and II for dendritic cell maturation and IL-12 production and their dependency on GM-CSF. *J. Immunol.* 169:3574.
28 Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J. and Paul, W. E. 1999. The IL-4 receptor: signaling mechanisms and biologic function. *Annu. Rev. Immunol.* 17:701.
29 Aversa, G., Punnonen, J., Cocks, B. G. *et al.* 1993. An interleukin 4 (IL-4) mutant protein inhibits both IL-4 or IL-13-induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptors. *J. Exp. Med.* 178:2213.
30 Kioi, M., Kawakami, K. and Puri, R. K. 2004. Mechanism of action of interleukin-13 antagonist (IL-13E13K) in cells expressing various types of IL-4R. *Cell. Immunol.* 229:41.
31 Geitjtenbeek, T. B., Torensma, R., van Vilet, S. J. *et al.* 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100:491.
32 Kato, M., Neil, T. L., Fearnley, D. B., McLellan, A. D., Vuckovic, S. and Hart, D. N. J. 2000. Expression of multilectin receptors and comparative FITC-dextran uptake by human dendritic cells. *Int. Immunol.* 12:1511.
33 Sallusto, F., Schaerli, P., Loetscher, P. *et al.* 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.* 28:2760.
34 D'Amico, G., Frascaroli, G., Bianchi, G. *et al.* 2000. Uncoupling of inflammatory chemokine receptors by IL-10: generation of functional decoys. *Nat. Immunol.* 1:387.
35 Langenkamp, A., Messi, M., Lanzavecchia, A. and Sallusto, F. 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat. Immunol.* 1:311.
36 Groux, H., Bigler, M., de Vries, J. E. and Roncarolo, M. G. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T cells. *J. Exp. Med.* 184:19.
37 De Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G. and de Vries, J. E. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174:1209.
38 Ria, F., Penna, G. and Adorini, L. 1998. Th1 cells induce and Th2 inhibit antigen-dependent IL-12 secretion by dendritic cells. *Eur. J. Immunol.* 28:2003.
39 Miller, M. J., Safrina, O., Parker, I. and Cahalan, M. D. 2004. Imaging the single cell dynamics of CD4+ T cell activation by dendritic cells in lymph nodes. *J. Exp. Med.* 200:847.
40 Miller, M. J., Hejazi, A. S., Wei, S. H., Cahalan, M. D. and Parker, I. 2004. T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. *Proc. Natl Acad. Sci. USA* 101:998.
41 Kobayashi, M., Azuma, E., Ido, M. *et al.* 2001. A pivotal role of rho GTPase in the regulation of morphology and function of dendritic cells. *J. Immunol.* 167:3585.
42 Wolfl, M., Batten, W. Y., Posovszky, C., Bernhard, H. and Berthold, F. 2002. Gangliosides inhibit the development from monocytes to dendritic cells. *Clin. Exp. Immunol.* 130:441.
43 Goxe, B., Latour, N., Chokri, M., Abastado, J. P. and Salcedo, M. 2000. Simplified method to generate large quantities of dendritic cells suitable for clinical applications. *Immunol. Invest.* 29:319.
44 Roberts, J. M., Yang, J. and Ronchese, F. 2004. IL-4 deficiency does not impair the ability of dendritic cells to initiate $CD4^+$ and $CD8^+$ T cell responses *in vivo*. *Int. Immunol.* 16:1451.
45 Menetrier-Caux, C., Thomachot, M. C., Alberti, L., Montmain, G. and Blay, J. Y. 2001. IL-4 prevents the blockade of dendritic cell differentiation induced by tumor cells. *Cancer Res.* 61:3096.